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Bioaugmentation of activated sludge with *Achromobacter denitrificans* PR1 for enhancing the biotransformation of sulfamethoxazole and its human conjugates in real wastewater: Kinetic tests and modelling

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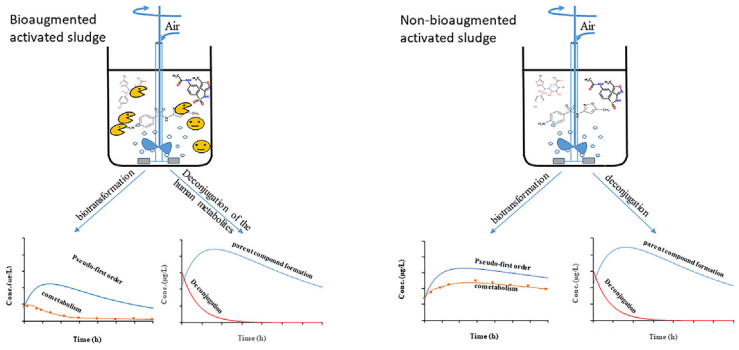
HIGHLIGHTS

- Activated sludge bioaugmented with *A. denitrificans* PR1 enhanced SMX transformation.
- Retransformation of human metabolites occurred under aerobic and anoxic conditions.
- *A. denitrificans* PR1 metabolised wastewater carbon sources as co-substrates.
- Co-metabolic models described well the biotransformation kinetics.

GRAPHICAL ABSTRACT



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ABSTRACT

Achromobacter denitrificans PR1 has previously shown potential to degrade the antibiotic sulfamethoxazole, whereby sulfamethoxazole biotransformation was stimulated in the presence of biogenic substrates. This study examined the biotransformation kinetics of sulfamethoxazole and its two main conjugates, N_4-acetyl-SMX and SMX-N_1-Glucuronide, by activated sludge and activated sludge bioaugmented with *A. denitrificans* PR1. SMX biotransformation under both anoxic and aerobic conditions was tested, with and without the addition of acetate as growth substrate, to understand the range of applicable conditions for bioaugmentation purposes. Biological process models, such as the pseudo-first order kinetic and cometabolic models, were also applied and, following the estimation of kinetic parameters, could well describe data measured in bioaugmented and non-bioaugmented AS batch experiments under various test conditions. Experimental and modelling results suggest that (i) retransformation of the two conjugates to SMX in AS occurred under both aerobic and anoxic conditions, and (ii) biotransformation kinetics of SMX can vary significantly depending on redox conditions, e.g., SMX was biotransformed by AS only under aerobic conditions. Notably, SMX biotransformation was significantly enhanced

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when PR1 was bioaugmented in AS. Addition of acetate as biogenic substrate is not necessary, as PR1 was capable of enhancing the SMX biotransformation by using the carbon sources present in wastewater. Overall, bioaugmentation by means of *A. denitrificans* PR1 could be a viable strategy for enhancing SMX removal in AS wastewater treatment plants (WWTPs).

1. Introduction

The intensive use of antibiotics for human and veterinary therapy has led to their continuous discharge, also in the form of conjugates, in the environment. WWTPs are not designed to remove these and other xenobiotic chemicals, and discharge of treated effluents into the environment has been a major concern due to the risk of a worldwide dispersal of antibiotic resistance genes [1].

Amongst antibiotics, sulfamethoxazole (SMX) is one of the most widely used synthetic sulfonamides worldwide. SMX enters WWTPs via human excretion in the forms of unchanged SMX (15–25% of the excreted dose) as well as the conjugated forms N_4-acetyl-SMX (Ac-SMX) (> 40%) and SMX-N_1-Glucuronide (SMX-Glu) (9–15%) [2]. The two human conjugates have been detected in wastewater influent and effluent, and were observed to rapidly deconjugate during wastewater treatment [3,4] which was considered to likely explain the reported 'negative removal' of SMX in wastewater treatment [3,5]. This suggests the importance of investigating the retransformation of the two major human conjugates to parent SMX, in order to explain the reported differences in removal efficiencies in WWTPs [6,7].

SMX removal has been shown to vary greatly, i.e. from negative (−138%) to very high (> 90%) [1] in full-scale WWTPs, and with variability in SMX biotransformation kinetics. SMX was also shown to be not readily biodegradable during the 28-day test period in a closed bottle test [8].

Biotransformation has been recognized as the major elimination mechanism of SMX and its conjugates during biological treatment of domestic wastewater, with minor contribution of sorption onto sludge (due to the polar nature of these compounds). Overall, literature reports of inconsistent and incomplete SMX elimination suggest that novel technologies/strategies would be required if more stringent discharge limits for SMX and other antibiotics are enforced. Bioaugmentation can be an alternative WWTP operational strategy to enable or enhance xenobiotics removal by inoculating specialized degrading bacteria [9]. Despite the fact that bioaugmentation has been studied for years in wastewater treatment to reinforce biological processes, few studies have tested the use of bioaugmentation for enhancing the removal of xenobiotics, e.g. 17β-estradiol [10], estradiol [11], fungicides [12]. With respect to antibiotics, bioaugmentation resulted in limited SMX removal when applying *Microbacterium* sp. strain BR1 in full-scale membrane bioreactors [13], except for SMX concentrations far higher than the ones normally found in municipal wastewater.

Previously, we showed that a pure culture of *Achromobacter denitrificans PR1* could exhibit faster biotransformation kinetics (up to two to three orders of magnitude higher) of SMX compared to AS alone [14], even at the low SMX concentrations typical of wastewater effluents. Given its ability to degrade SMX in the presence and/or absence of other additional carbon sources (acetate and succinate) at environmentally relevant concentrations (typical of e.g., wastewater effluents), the strain likely has potential for treating SMX in wastewater upon bioaugmentation. Therefore, the overall objective of this work was to investigate whether PR1 can enhance SMX biotransformation kinetics when bioaugmented to AS with real wastewater feed. Specifically, we (i) investigated the effect of redox conditions, i.e. aerobic and anoxic conditions, on the transformation rates of targeted compounds; (ii) assessed the potential influence of retransformation processes of the two main human conjugates, i.e. Ac-SMX and SMX-Glu, on the fate of sulfamethoxazole under the testing conditions; and (iii) evaluated the need for supplementation with a biogenic substrate (e.g. acetate) or whether the availability of carbon sources in wastewater could serve as biogenic substrates to achieve a sufficiently interesting kinetic for SMX removal upon bioaugmentation of AS with PR1. Modelling the fate of xenobiotics in WWTPs can be a useful tool to understand their removal mechanisms, predict and reduce their emissions with treated effluent through process optimization. Specifically, the Activated Sludge Modelling framework for Xenobiotics (ASM-X), has been previously used to predict the fate of SMX in biological treatment systems [7] and to identify factors (influent concentration of conjugates, solid residence time) possibly explaining the variability in SMX removal efficiencies [15]. In this context, suitable mathematical models were developed to examine the metabolic mechanism and predict kinetics of SMX and human conjugates biotransformation upon bioaugmentation of *A. denitrificans* PR1 into AS.

2. Materials and methods

2.1. Chemicals and reagents

Reagent grade (purity ≥ 99%) SMX was purchased from Sigma-Aldrich. Ac-SMX, SMX-Glu and isotopically labelled Ac-SMX-d4, SMX-d4-Glu, SMX-d4 were obtained from Toronto Research Chemicals, Inc. (TRC, Canada). Individual stock standard solutions were prepared on a

Table 1
Overview of the different tested conditions in the bioaugmented and non-bioaugmented AS experiments.

		Batch	Feed	AS (gTSS L^{-1})	*A. denitrificans* PR1 ($g_{biomass}$ L^{-1})	Acetate ($mg_{COD}L^{-}$)	NaN_3 ($mg_{azide}g^{-1}_{TSS}$)	ATU (mg L^{-1})
Control	Control 1	C1	MilliQ-water	–	–	–		
	Control 2	C2	WW*	~3	–	–	~650	
Nitrification-inhibition		ATU	WW*	~3	–	–		30
		Without ATU	WW*	~3	–	–	–	–
Bioaugmentation tests	Aerobic	A1	WW*	~3	0	0		
		A2	WW*	~3	0	137–152		
		A3	WW*	~3	~0.05–0.06	0		
		A4	WW*	~3	~0.05–0.06	137–152		
	Anoxic	An1	WW*	~3	0	0		
		An2	WW*	~3	~0.05–0.06	0		

WW*: wastewater from the effluent of a primary sedimentation tank was centrifuged at 10,000*g* for 15 min at 4 °C, and then filtered through Whatman® Glass microfiber filters, pore size 1.2 µm binder free, Grade GF/C before feeding to the reactors.

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